



Molecular phylogeny diagnosis of *Echinococcus granulosus* sensu lato and *Taenia hydatigena* determined by mitochondrial *Cox1* and *SSU-rDNA* markers in Iranian dogs: Indicating the first record of pig strain (G7) in definitive host in the Middle East

Seyed Reza Mirbadie^{a,b}, Abbas Najafi Nasab^c, Mohammad Ali Mohaghegh^{d,e}, Pirasteh Norouzi^a, Mehdi Mirzaii^f, Adel Spotin^{g,h,*}

^a School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

^b Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran

^c Social Security Organization, Semnan, Iran

^d Department of Laboratory Sciences, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran

^e Health Sciences Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran

^f Department of Microbiology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

^g Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

^h Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Unawareness of canine parasitic diseases among at-risk hosts and an uncontrolled program of stray dog population have caused that zoonotic parasites received great attention in endemic regions of the Middle East. A total of 552 faecal samples were collected between December 2016 to January 2018 from stray (n = 408) and domestic (n = 144) dogs of Iran. All specimens were coproscopically observed following concentration and flotation techniques. Subsequently, the DNAs of taeniid eggs were extracted, amplified, and sequenced by targeting of mitochondrial cytochrome oxidase subunit 1 and small-subunit ribosomal DNA markers. The overall prevalence of canine intestinal parasites found 53.6%. The following parasites and their total frequencies were identified: taeniid (10.5%), *Dicrocoelium dendriticum* (0.7%), *Trichuris vulpis* (1.2%), *Capillaria* spp. (2.3%), *Blastocystis* spp. (5.2%), *Ancylostoma* spp. (2%), *Eimeria* spp. (13.2%), *Dipylidium caninum* (2.3%), *Toxocara canis* (3.8%), *Giardia* spp. (8.5%), and *Toxascaris leonina* (3.6%). Stray dogs were characterized more likely to be poliparasitized and indicated a higher prevalence of taeniid (10.9%), *T. canis* (4.4%) *Giardia* spp. (10.1%) than domestic dogs ($P > 0.05$). Phylogenetic and sequence analysis of *Cox1* and *SSU-rDNA* indicated a low genetic diversity (Haplotype diversity; 0 to 0.495) in *E. granulosus* sensu lato G1, G3, G7 genotypes, and *Taenia hydatigena*. The pairwise sequence distances between G7 isolates showed an intra-diversity of 0.7%–1.5% and identity of 98.5%–100%. The first occurrence of pig strain (G7) from Iranian dogs might have substantial implications in the drug treatment of infected dogs due to the shorter maturation time of G7 compared with G1 genotype. Thus, the preventive strategies should be noticed to determine the risk factors, the importance of applying the hygienic practices, and well adjusting deworming programs for the Iranian dogs and at-risk individuals.

1. Introduction

Zoonotic enteric parasitic diseases remain a public health concern among at-risk populations, particularly immunocompromised individuals due to close proximity with domestic and wild animals in developing countries [1]. Among the threatening animal reservoirs,

stray dogs have received great attention due to their zoonotic potential in maintenance, transmission, and epizootiology of zoonotic diseases including cystic echinococcosis (CE) or toxocarosis [2]. The population of domestic dogs is more than 700 million in the worldwide, which most of them (~75%) are attributed to un-owned dogs [3,4]. The ration of dogs to humans estimated to be between 10 to 33 dogs per 100

* Corresponding author at: Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail address: Spotina@tbzmed.ac.ir (A. Spotin).

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residents [5]. The CE as a neglected orphan disease threatens various numbers of intermediate hosts including livestock spp. and humans [6,7]. CE is one of the 17 neglected serious diseases in the worldwide with over 1 million cases of human infection at any time [8]. The infection rate of stray dogs with *E. granulosus* indicates a significant range of 5%–49% in various regions of Iran [9]. One of the most important diagnostic challenges in the differentiation of family Taeniidae (especially *Echinococcus* spp.) is the similarity of their eggs, in which they are microscopically indistinguishable [10]. Furthermore, microscopic observation does not allow a reliability estimate of infection intensity (parasitic load) among the suspected hosts. Currently, a wide range of conventional molecular testing strategies are being used to estimate the *Echinococcus* burdens in definitive hosts including necropsy, copro-antigens enzyme-linked immunosorbent assays (ELISA), sedimentation and counting technique, intestinal scraping technique (IST), copro-DNA molecules by polymerase chain reaction (PCR), and Real-Time PCR [6,11–13]. The *E. granulosus* copro-antigen detection ELISA with a sensitivity of 60% (specificity 69%) and IST method with a sensitivity of 78%, have been employed worldwide to monitor the dog infection in control program [14,15]. On the one hand, the copro-PCR technique has been broadly used as a reliable method to identify the *Echinococcus* spp. in both stray and domestic dogs in different regions of Iran and other countries [16–18]. Despite the occurrence of unexpectedly large proportion of *E. granulosus* in stray dogs (80% by necropsy) [19] knowledge on the genetic diversity of circulating *Echinococcus* spp. in the dog faecal samples of Iran has not fully understood yet [20]. The current phylo-molecular study was aimed to ascertain the canine gastrointestinal parasite prevalence and heterogeneity features of family Taeniidae in a hyperendemic Middle East focus, Iran to integrate their genetic variation data.

2. Materials and methods

2.1. Sample collection and coprological examination

A total of 552 faecal samples were collected in the time period of December 2016 to January 2018 from stray (n = 408) and domestic (n = 144) dogs of different parts in Iran, including Shahrood, Damghan, Semnan, and Garmsar cities. 10–20 g of the fresh faecal sample from each domestic dog with the help of the household family members, were collected in a labeled sterile clinical bottle. Stray dog's samples were collected from places where a lot of stray dogs were present around the cities. To minimize and avoid repeated sampling from the same dog, only very fresh samples were collected at a distance of about 150–200 meters. Faecal samples (n = 552) were analyzed to observe the taeniid eggs (*Echinococcus* spp./*Taenia* spp.) and enteric parasites using conventional techniques (Sedimentation and flotation). The details of the collection of faecal samples in different regions of Iran are given in Table 1. To inactivate taeniid eggs and avoid the risk of laboratory infection all samples were kept at -80°C for 5–8 days and then were frozen at -20°C until DNA extraction [21]. All specimens were coprologically identified following formalin–ether concentration

Table 1

The prevalence of *Taenia/Echinococcus granulosus* sensu lato eggs in faecal samples of stray and domestic dogs by microscopic observation and copro-PCR technique based on under studied cities.

Study areas	No. of examined faecal samples	Source of dogs		Microscopic observation No. of taeniid eggs (%)	Copro-PCR No. of <i>Taenia hydatigena</i> / <i>E. granulosus</i> sensu lato (%)
		Stray	Domestic		
Iran	Mayamey	51	32	19	6 (7.84)
	Shahrood	186	131	55	19 (7.52)
	Damghan	102	86	16	9 (5.88)
	Semnan	124	85	39	14 (7.25)
	Garmsar	89	70	19	10 (8.98)
	Total	552	404	148	58 (10.5)

(sedimentation) and zinc sulphate floatation techniques (ZnSO₄ solution, 1.18 specific gravity) [22].

2.2. DNA extraction and polymerase chain reaction

A total of 41 DNA samples were successfully extracted from positive taeniid eggs and 15 microscopically negative samples were randomly amplified by PCR to evaluate the accuracy of the microscopic assessment. Subsequent to freeze-thawing technique, the suspension was combined with an equal volume of glass beads and then was vortexed vigorously for 8–10 min. Following adding proteinase K, the suspension was incubated at 60°C for one overnight. DNA was extracted using a standard extraction procedure by using the QIAamp fast DNA Stool Mini kit (Cat.no.69504, QIAGEN, and Germany) according to the manufacturer's instructions with minor modifications. The DNA concentration of each extraction was measured using a Nanodrop (Thermo Scientific Inc., Wilmington, DE). The eluted DNA was kept at -20°C until molecular analysis. In this study, primers JB3/JB4.5 (cytochrome c oxidase subunit 1 (*Cox1*) and EGfor1/EGrev1 (small-subunit ribosomal DNA (*SSU-rDNA*) of family Taeniidae were amplified by targeting of sequences of mitochondrial DNA with total lengths of 444bp and 149bp, respectively. The oligonucleotide sequences, PCR reactions, and annealing temperatures have been previously described [16,23].

2.3. DNA sequencing, diversity indices, pairwise sequence distances, phylogenetic analysis, and haplotype network

Amplicons of positive taeniid eggs (n = 32) were randomly selected, purified, and sequenced (Bioneer. Company, Korea) by targeting *Cox1* and *SSU-rDNA* in both directions using the JB3/JB4.5 and EGfor1/EGrev1 primers. The ambiguity sites of sequences were edited according to reference sequence (RefSeq) using the Sequencher™v.4.1.4 software based on IUPAC codes. To assess the genetic diversity, the DnaSP software [24] was used according to the analysis of molecular variance to calculate the haplotype (genetic) diversity (Hd); nucleotide diversity, (π); number of haplotypes (Hn). A pairwise sequence distance matrix was computed using the Meg Align program to show the percent identity (%) and intra-inter diversity levels among geographical sequences of the *Cox1* and *SSU-rDNA* genes. To authenticate genetic associations among identified genotypes of *Echinococcus* spp. inferred by the *Cox1* gene, a phylogenetic tree was generated by Splits Tree 4.0 software with Neighbor-Net algorithm. The distance scale was estimated 0.01. *Taenia hydatigena* was addressed as an out-group branch. Multiple alignments among the amino acid sequences were done based on ClustalW method (BioEdit software, version 7.0.5). To show the genealogical relationships at intra-genetic diversity of identified *E. granulosus* s.l. a haplotype network was constructed by PopART software using the Median Joining algorithm.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS 18 Pearson's Chi-

Table 2
Prevalence of gastrointestinal parasitic infections by microscopic observation among different sources of dogs in Iran.

Parasite (Genus/species)	Source of dogs		Total (n = 552) (%)
	Domestic dogs (n = 148)(%)	Stray dogs (n = 404)(%)	
Taeniidae (<i>Taenia hydatigena</i> and <i>E. granulosus sensu lato</i>)	14(9.4)	44(10.9)	58(10.5)
<i>Dicrocoelium dendriticum</i>	1(0.6)	3(0.7)	4(0.7)
<i>Trichuris vulpis</i>	2(1.3)	5(1.2)	7(1.2)
<i>Capillaria</i> spp.	4(2.7)	9(2.2)	13(2.3)
<i>Blastocystis</i> spp.	9(6)	20(4.9)	29(5.2)
<i>Ancylostoma</i> spp.	3(2.7)	8(1.9)	11(2)
<i>Eimeria</i> spp.	20(13.5)	53(13.1)	73(13.2)
<i>Dipylidium caninum</i>	3(2.7)	10(2.4)	13(2.3)
<i>Toxocara canis</i>	3(2.7)	18(4.4)	21(3.8)
<i>Giardia</i> spp.	6(4)	41(10.1)	47(8.5)
<i>Toxascaris leonina</i>	5(3.3)	15(3.7)	20(3.6)

Square test was used to compare the significant association for the prevalence of intestinal parasites and different those in dogs studied. A *P* value of less than 0.05 was set as statistically significant.

3. Result

3.1. Coprological examination, PCR, and sequence analyses

The overall prevalence of taeniid eggs in faecal samples of stray and domestic dogs was identified by coproscopical and copro-PCR techniques (Table 1). The higher prevalence rate was obtained in Garmsar (8.98%) followed by Mayamey (7.84%), Shahrood (7.52%), Semnan (7.25%) and Damghan (5.88%) cities. The prevalence of taeniasis/echinococcosis was 7.42% (Table 1). Faecal examination showed 296 (53.6%) out of 552 dogs including 226 of 404 stray dogs (55.9%) and 70 of 148 domestic dogs (47.2%) were positive with at least one species of parasites.

The most frequently enteric helminthic and protozoan infections belonged to *Eimeria* spp. (13.2%) and *Taenia* spp. (10.5%), respectively (Table 2). Coprological findings to illustrate the enteric parasites listed in Table 2. The following parasites and their whole frequencies in stray and domestic samples were identified as; taeniid (10.5%), *Dicrocoelium dendriticum* (0.7%), *Trichuris vulpis* (1.2%), *Capillaria* spp. (2.3%), *Blastocystis* spp. (5.2%), *Ancylostoma* spp. (2%), *Eimeria* spp. (13.2%), *Dipylidium caninum* (2.3%), *Toxocara canis* (3.8%), *Giardia* spp. (8.5%), and *Toxascaris leonina* (3.6%). Stray dogs were characterized more likely to be poliparasitized and indicated a higher relative prevalence of taeniid (10.9%), *T. canis* (4.4%), and *Giardia* spp. (10.1%) than domestic dogs (Table 3), however, this difference was not statistically significant ($P > 0.05$). Based on coproscopical technique 10.5% (n = 58) of faecal isolates were identified to taeniid eggs, while PCR prevalence (using two species-specific primer pairs; *Cox1* and *SSU-rDNA*) for *E. granulosus* s.l. and *T. hydatigena* was corresponded to 6.52% (n = 36) and 0.9% (n = 5) respectively. The number of single and multiple

Table 3

The prevalence of *Taenia hydatigena* and *Echinococcus granulosus sensu lato* eggs in faecal samples of stray and domestic dogs by microscopic observation and copro-PCR technique.

Type of dog	No. of examined samples (%)	Microscopic observation No. of taeniid eggs (%)	Copro-PCR No. of <i>Taenia hydatigena</i> and <i>E. granulosus sensu lato</i> (%)
Domestic dogs	148 (26.8)	14 (9.4)	7 (4.7)
Stray dogs	404 (73.1)	44 (10.9)	34 (8.4)
Total	552	58	41

parasitic infections in dogs are shown in Table 4. Dogs harbouring two parasites were more common (17.8%) than those harbouring one (14.7%), three (12.3%) or four (7%) and > four (1.8%) (Table 4).

The fragments of 444bp and 149bp were successfully amplified by targeting *Cox1* and *SSU-rDNA* genes, respectively (Fig. 1; A, B). According to sequence analysis of *Cox1* and *SSU-rDNA*, 68.75%, 6.25%, 15.63%, and 9.4% isolates were explicitly found to correspond to *E. granulosus* G1, *E. granulosus* G3, *E. canadensis* G7 genotypes (isolated from both stray and domestic dogs), and *T. hydatigena*, respectively (Table 5). No mixed infections with *Echinococcus* spp. were found among all sequence analyses.

3.2. Multiple sequence alignment, diversity indices, and pairwise sequence distance matrix

Based on multiple sequence alignments of *Cox1* and *SSU-rDNA*, 12 (G1 genotype) one (G3 genotype), two (G7 genotype; Submitted to the GenBank database under Accession number; MK256482), and two (*T. hydatigena*) new haplotypes (mainly transition or transversion mutations) were unambiguously identified (Table 5). The diversity indices of *E. granulosus* s.l. G1 and G3, and G7 genotypes are given in Table 5. Heterogeneity analysis of G1, G3, and G7 sequences were shown a lack (G3: Hd: 0.00) to low (G1; Hd: 0.420–0.495, G7: Hd: 0.414) genetic (haplotype) diversity (Table 5). In our targeted regions of *Echinococcus* DNA, insertion or deletion (*Indel*) mutations were not found in *E. granulosus sensu stricto* (s.s.) (G1 and G3) and *E. canadensis* G7. The multiple amino acid alignments of the G7 genotype (*Cox1*; Sem3* and Sem4*) in comparison to globally G7 RefSeqs (France; Accession nos: MH301008-MH301009, MH301010, MH301012, MH301015 and Italy; Accession nos: MH301016, MH301018- MH301019) indicated the occurrence of three non-synonymous substitutions at the codons 134, 135, and 136, where Lysin (L) replaced a Methionine (M), Phenylalanine (F) replace a Serine (S), and F replaced a L, respectively (Fig. 2). The pairwise sequence distances between identified G7 genotype (*Cox1*; Sem3* and Sem4*) and GenBank RefSeq database (above mentioned) showed an intra-diversity of 0.7%–1.5% and identity of 98.5%–100% (Fig. 3), while the G7 sequence distance analysis of *SSU-rDNA* showed a 100% intra-diversity and 100% identity with the *E. canadensis* (G7) GenBank RefSeq under accession number MH301010 (Pig strain, France).

3.3. Phylogenetic tree and haplotype network

To discern the taxonomic status of taeniid isolates, (*E. granulosus* G1:Sem1*-Sem2*, G3: Sem3* G7: Accession no; MK256482, Sem4*-Sem5* and *T. hydatigena**) a Neighbor-net phylogenetic tree was constructed inferred from *Cox1* sequences. The clade topology of *Echinococcus* spp. shown that the *E. canadensis* G7 (Sem4*-Sem5*) and *E. canadensis* G6 sequences placed in a same cluster namely, *E. intermedius* complex (Fig. 4). The genealogical relationship among identified haplotypes of G1, G3, and G7 (Accession no; MK256482) genotypes (marked by asterisk*) was constructed based on *Echinococcus* s.l. *Cox1* sequences (Fig. 5).

Table 4
Number of single and multiple parasitic infections in dogs in Iran.

Parasite (Genus/species)	Number of parasite in total dogs (%)					Total
	1	2	3	4	> 4	
Taeniidae (<i>Taenia hydatigena</i> and <i>E. granulosus sensu lato</i>)	11 (19)	9 (15.5)	18 (31)	14 (24.1)	6 (10.4)	58 (100)
<i>Dicrocoelium dendriticum</i>	0 (0)	1 (25)	2 (50)	1 (25)	0 (0)	4 (100)
<i>Trichuris vulpis</i>	0 (0)	1 (14.3)	3 (42.8)	2 (28.6)	1 (14.3)	7 (100)
<i>Capillaria</i> spp.	0 (0)	2 (15.4)	4 (30.8)	6 (46.1)	1 (7.7)	13 (100)
<i>Dipylidium caninum</i>	2 (15.4)	4 (30.8)	3 (23)	4 (30.8)	0 (0)	13 (100)
<i>Ancylostoma</i> spp.	1 (9)	3 (27.4)	4 (36.4)	2 (18.2)	1 (9)	11 (100)
<i>Toxascaris leonina</i>	3 (15)	7 (35)	6 (30)	3 (15)	1 (5)	20 (100)
<i>Toxocara canis</i>	3 (14.3)	5 (23.8)	8 (38.1)	3 (14.3)	2 (9.5)	21 (100)
<i>Eimeria</i> spp.	6 (8.3)	18 (24.6)	25 (34.2)	21 (28.8)	3 (4.1)	73 (100)
<i>Giardia</i> spp.	5 (10.6)	19 (40.4)	13 (27.7)	8 (17.1)	2 (4.2)	47 (100)
<i>Blastocystis</i> spp.	4 (13.8)	6 (20.7)	13 (44.8)	5 (17.2)	1 (3.5)	29 (100)
Total number of dogs (%)	81 (14.7)	98 (17.8)	68 (12.3)	39 (7)	10 (1.8)	552 (100)

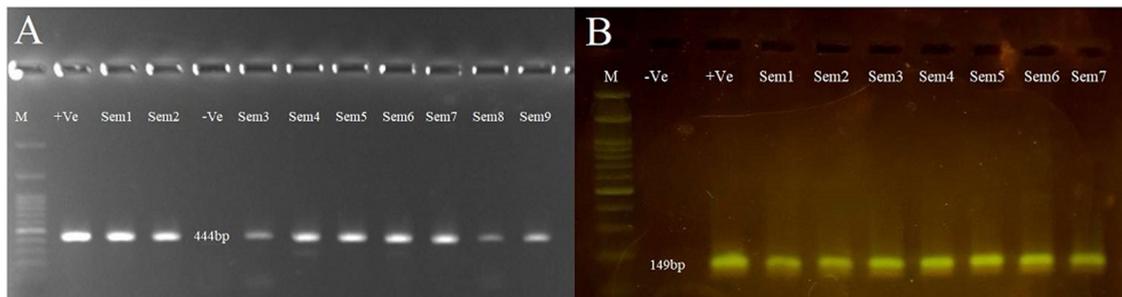


Fig. 1. A-Single round PCR assay by targeting *Cox1* to amplify *Echinococcus* spp. and *Taenia* spp. (444bp); Lanes: Sem3 and Sem4, Sem6-Sem12. Positive isolate: Lane 2 (+Ve; Positive control), Lane 5 (-Ve: Negative control), M: 100 bp DNA ladder marker. B- Single round PCR assay by targeting *SSU-rDNA* to amplify *Echinococcus* spp. (149bp); Lanes: Sem1-Sem7.

Table 5

Diversity indices of *Echinococcus granulosus* G1/G3 genotypes, *E. canadensis* genotype G7, and *Taenia hydatigena* based on cytochrome c oxidase subunit 1 and mitochondrial small-subunit rDNA sequences. N: number of isolates; Hn: number of haplotypes; Hd: haplotype (gene) diversity; Nd: nucleotide diversity.

Region	Parasite (%)	Gene	Diversity indices					
			N	Hn	Hd ± SD	Nd (π)	No. of polymorphic sites	
Iran	<i>Echinococcus granulosus sensu lato</i>	<i>Echinococcus granulosus</i> genotype G1 (68.75)	<i>Cox1</i>	8	2	0.429 ± 0.169	0.00224	2
			Small-subunit rDNA	14	10	0.495 ± 0.151	0.03114	4
		<i>Echinococcus granulosus</i> genotype G3 (6.25)	<i>Cox1</i>	2	1	0 ± 0.400	0.00000	0
			Small-subunit rDNA	ND	ND	ND	ND	ND*
		<i>Echinococcus intermedius</i> genotype G7 (15.63)	<i>Cox1</i>	3	1	0.414 ± 0.500	0.00000	0
			Small-subunit rDNA	2	1	0 ± 0.500	0.00000	0
	<i>Taenia hydatigena</i> (9.4)	<i>Cox1</i>	3	2	0.667 ± 0.314	0.00345	2	
	Total		32					

ND: Not determined.

4. Discussion

Unawareness of zoonotic diseases amongst at-risk individuals and an uncontrolled program of stray and domestic dogs roaming in human environments, chances of getting the parasitic diseases have remarkably augmented in some neglected hyperendemic regions of Middle East mostly Iran [9,25,26]. In this study, the prevalence of canine gastrointestinal parasites was found to correspond to 53.6%, particularly in stray dogs (55.9%), indicating an excessive infection rate that necessitates launching an effective de-worming regime, monitoring, and preventive strategies in Iran. According to the earlier study conducted in the same areas, which estimated an 80% prevalence of enteric parasites in faecal samples of stray dogs in Garmsar city [19]. Additionally, the prevalence of hydatidosis in the slaughtered animal and human estimated 13.3% and 8.6%, respectively in Semnan province [27]. The global expansions of gastrointestinal parasites in faecal

samples of various sources of dogs ranged from 20.4%–84.78% (Table 6) [28]. These discrepancies might be explained by diagnostic techniques, sampling protocols, anthelmintic usage, patterns of offal disposal, the breed of dog population, geographical location, and existence of stray dogs that may have been more greatly involved than those included in current investigation [29–31]. In this study, stray dogs were found more likely to be poliparasitized and indicated a higher relative prevalence of taeniid (10.9%) than domestic dogs. This significant infection rate of stray dogs with taeniasis/echinococcosis can be followed by several facts; Given that the killing and/or euthanasia of stray dogs have prohibited by Iranian policymakers, their abundance in rural areas around the abattoir where infected carcasses from slaughtered are easily achievable to stray dogs, as well diet type or/and food behaviors are the most effective to infection compared to domestic dogs. In addition, trapping, de-worming regimes, and management of domestic dogs in urban areas are easier than stray dogs in

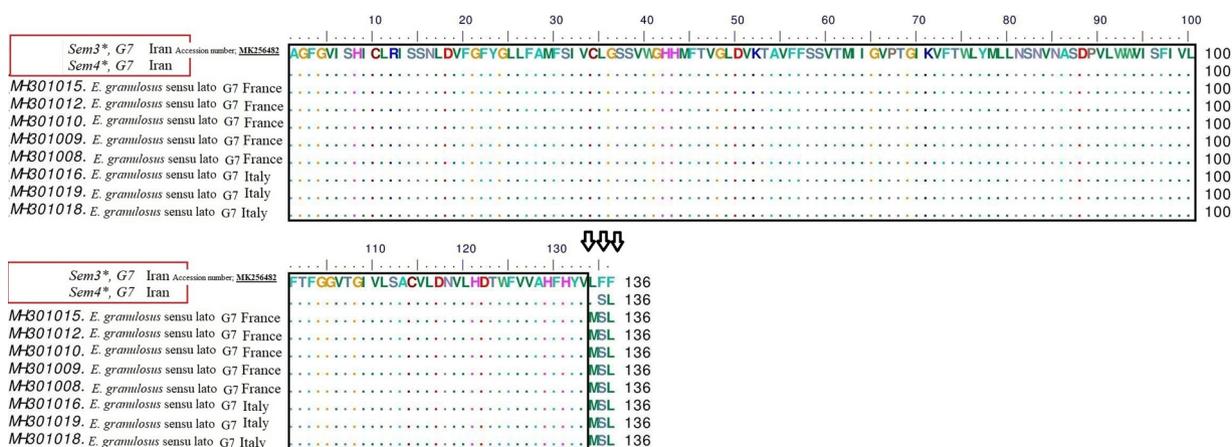


Fig. 2. The multiple amino acid alignments of the *E. canadensis* G7 genotype *Cox1* (Sem3* and Sem4*) in comparison to globally G7 RefSeqs (France and Italy).

around the cities. Due to the shortage of well-documented records on the *E. granulosus* s.l. genotypes originating from stray and domestic dogs in the Iran, the current study provides the first inclusive strain genotyping of dog isolates in this region. The four distinct strains of family Taeniidae (*E. granulosus* s.l. G1, G3, G7 genotypes, and *T. hydatigena*, Hd; 0 to 0.429–0.495) were unambiguously revealed by sequencing and phylogenetic analyses. The findings presented that sheep strain (G1; 68.75%) was the most dominant genotype of CE in both stray and domestic dogs of Iran, where confirmed by several researchers in endemic regions of Iran [20]. In a similar study, the *E. canadensis* G6 genotype had been previously reported from one camel isolate in a same province (Semnan) [27], interestingly no camel strain (G6) was found in our positive *Echinococcus* sequences. This may be due to scarifying of camels in industrial abattoirs which resulted in inaccessibility of stray dogs to infected viscera to protoscoleces. Additionally, we firstly isolated and reported the G7 genotype (submitted to GenBank, Accession no: MK256482) in five dog isolates (15.63%) from Iran, where this strain (G7) had previously identified by PCR-RFLP and sequencing in Iranian goats of Northeast Iran [32]. The occurrence of the G7 strain in Iranian stray/domestic dogs has substantial implications to employ the monitoring programs of human hydatidosis such as regular de-worming of infected dogs due to the shorter maturation time of G7 genotype in comparison with the sheep strain (G1) [33].

On the one hand, the identification of pig strain (G7) from stray dogs of Central Iran highlights the specific affinity of the *E. intermedium* cluster (G6–G7) to definitive host, since *E. canadensis* G6 genotype (Camel strain) had been formerly reported from one stray dog in Northwest of Iran [9]. According to this issue, we postulate that Central-Northeast to Northwest of Iran may serve interesting regions for the diversification and evolution of *E. intermedium* clade (G6/G7) in infected stray dogs. *E. canadensis* G7 genotype isolated from intermediate hosts (Egypt; human isolate and Turkey; sheep isolate) has been sporadically reported from some countries of the Middle East region [34,35].

However, a recent comprehensive study demonstrated that the sequencing of complete mitogenomes provides highly better phylogeny insights on the differentiation of *E. granulosus* s.l. genotypes G6 and G7 compared with the commonly used *Cox1* gene [36]. Therefore, based on our knowledge, current evidence provides the first record of pig strain in definitive hosts (stray/domestic dogs) isolated from Middle East region [36].

Lymbery et al., have shown that the *E. canadensis* G6 and G7 genotypes (unlikely G8 and G10 genotypes) have a largely allopatric (occurrence or parasite populations in separate regions) distribution in the world [37]. In this study, the occurrence of low genetic diversity of G1 (Hd: 0.420–0.495), G3 (Hd: 0.00), and G7 (Hd: 0.414) genotypes circulating among the all dog’s isolates may probably justify by either the self-fertilization of the hermaphroditic adult worms or the longevity of the parasite in Canids which is potentially reduced the degree of genetic heterogeneity of mitochondrial genomes (*Cox1/SSU-rDNA*) in local *Echinococcus* populations [38]. However, genetic diversity of *E. granulosus* s.s. genotype G1 on a global scale using near-complete mitogenome sequences shown a high genetic diversity in both intermediate and definitive hosts [39]. In this study, a moderate genetic diversity of *T. hydatigena* (Hd: 0.667) was estimated by analyzing of *Cox1* sequences. Nevertheless, a recent study conducted by Boufana et al. (2015) shown that goat *Cysticercus tenuicollis* isolates (Hd: 0.947) were genetically different from adult *T. hydatigena* isolates (Hd: 0.911) from Sardinian sheep [40].

This study describes the first description of *E. canadensis* G7 genotype in stray and domestic dogs of Iran. The occurrence of this rare strain in the various sources (stray/domestic dogs) can potentially enhance the contamination rate of the susceptible intermediate hosts to interfere with G7 genotype in both rural and urban areas. Furthermore, the presence of remarkable non-synonymous substitutions at the codons 134, 135, and 136 of G7 genotype can be addressed in the taxonomic status of parasite and emerging of possible drug-resistance alleles in clinical isolates.

		Percent Identity											
		1	2	3	4	5	6	7	8	9	10		
Divergence	1	■	99.3	98.5	98.5	98.5	98.5	98.5	98.5	98.5	98.5	1	Sem3*, G7 Sem4*, G7 Accession number: MK256482
	2	0.7	■	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	2	
	3	1.5	1.0	■	100.0	100.0	100.0	100.0	100.0	100.0	100.0	3	
	4	1.5	1.0	0.0	■	100.0	100.0	100.0	100.0	100.0	100.0	4	
	5	1.5	1.0	0.0	0.0	■	100.0	100.0	100.0	100.0	100.0	5	
	6	1.5	1.0	0.0	0.0	0.0	■	100.0	100.0	100.0	100.0	6	
	7	1.5	1.0	0.0	0.0	0.0	0.0	■	100.0	100.0	100.0	7	
	8	1.5	1.0	0.0	0.0	0.0	0.0	0.0	■	100.0	100.0	8	
	9	1.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	■	100.0	9	
	10	1.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	■	10	
		1	2	3	4	5	6	7	8	9	10		

Fig. 3. The pairwise sequence distances between identified G7 genotype (*Cox1*; Sem3* and Sem4*) and GenBank RefSeq database.

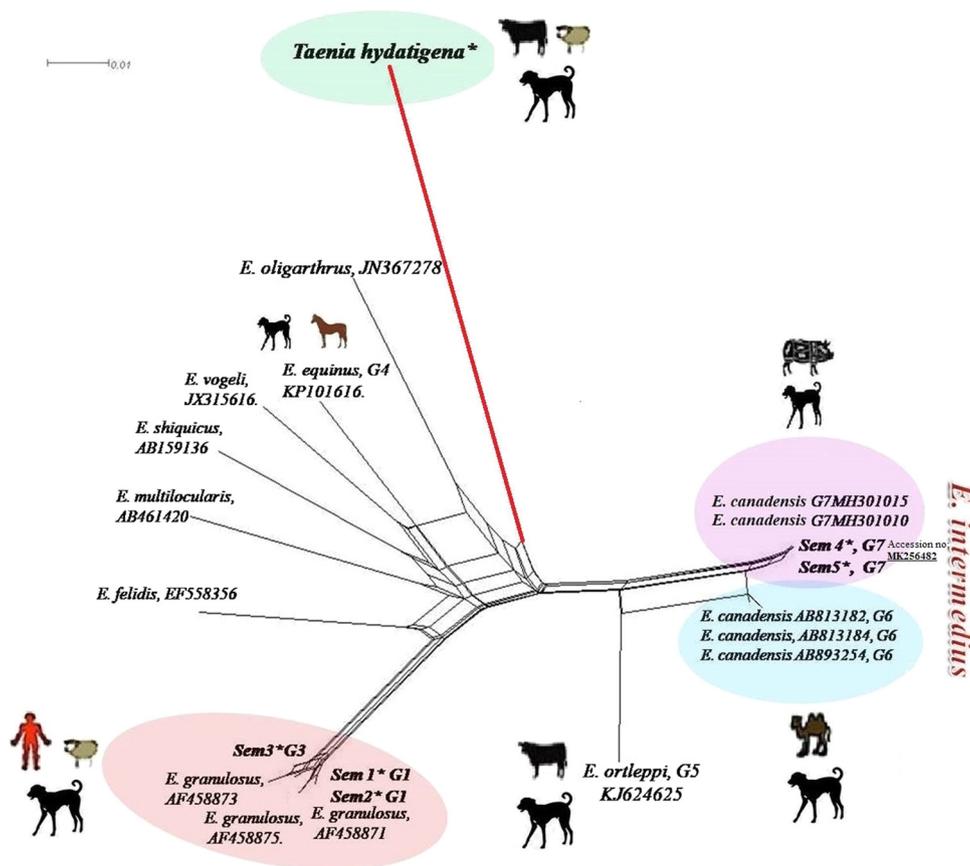


Fig. 4. Neighbor-Net graph drawn by different genotypes of *E. granulosus* sensu lato by using the Splits Tree 4.0 program. The identified isolates (G1, G3, G7 genotypes, and *Taenia hydatigena*) marked by asterisk (*).

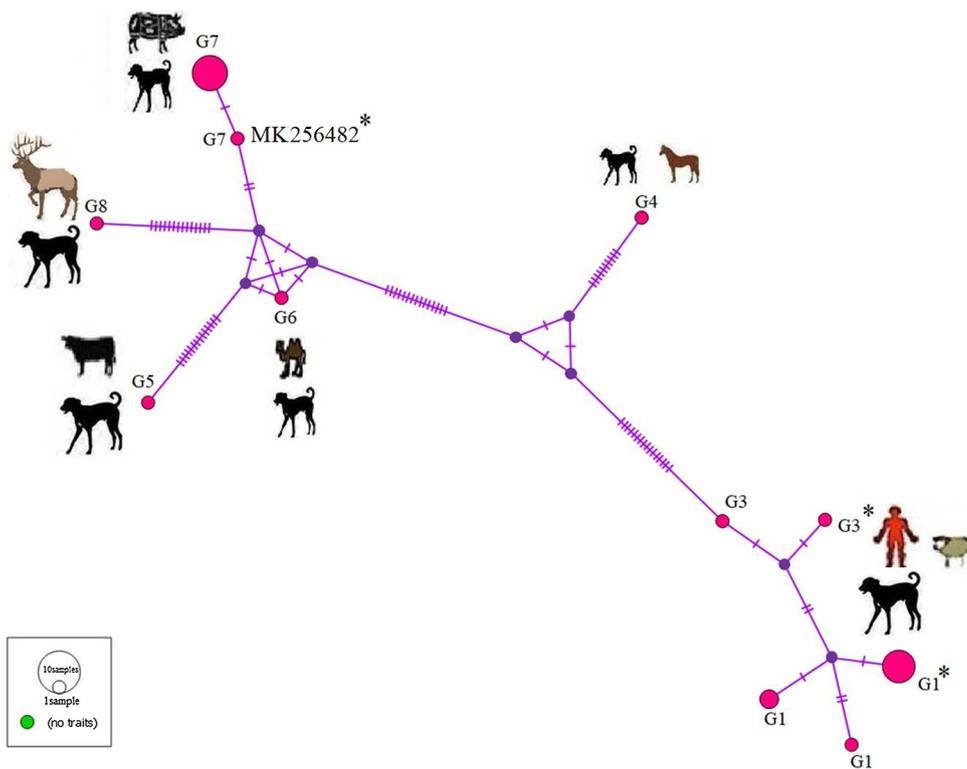


Fig. 5. Median Joining haplotype network of *Echinococcus granulosus* sensu lato genotypes of *Cox1* sequences obtained from Iran. Identified haplotypes (G1, G3, and G7) in the present study marked by asterisk (*). The red circles are relative to the frequency of each haplotype. Violet circles represent the hypothetical haplotypes. Each line between haplotypes indicate single mutational step.

Table 6

The global prevalence of intestinal parasites in faecal samples of various sources of dogs obtained from different countries.

Country/City	No. of faecal samples	Source of dogs	Prevalence (%)	Reference
Western Iran/ Kermanshah	301	Domestic and stray dogs	76.4	[41]
Western Iran/Hamadan	1500	Household and stray dogs	20.4	[42]
Iran	77	Domestic and Stray dogs	66.0	[43]
Western Iran	210	Pet dogs	6.7	[44]
Iran/Garmsar	50	Stray dogs	80	[19]
Iran	100	Domestic dog	57	[45]
Iran	80	Stray dogs	80.0	[46]
Greece	281	Shepherd and hunting dogs	26.0	[47]
Ethiopia	46	Stray dogs	84.78	[48]
	384	Pet dogs	75.26	
Italy	239	Owned dogs	31.0	[49]
Island	97	Owned dogs	71.4	[50]
Italy	150	Shelter and kennel dogs	86	[51]
Brazil	138	Stray dogs	73.6	[29]
		Domiciled dogs	34.4	
Italy/Sardinia	300	Farm dogs	taeniid eggs, 8.3	[52,53]

5. Conclusions

Current results indicated that the low genetic diversity of *E. granulosus* s.l. G1, G3, G7, and *T. hydatigena* are being unambiguously circulated among the infected dogs of Iran. The first occurrence of pig strain (G7) in Iranian dog has considerable implications in the drug treatment of infected dogs and controlling of plausible clinical isolates. The circulation of an unexpectedly large proportion of canine zoonotic parasitic infections in Iran can consider as a potential source of threatening pathogens to dog owners and accidental hosts' health that have unintentionally contact with their faecal samples. Thus, the preventive strategies should be noticed to determine the risk factors, the importance of applying the hygienic practices, and well adjusting deworming programs for the Iranian dogs and at-risk individuals. Further exploration will be indispensable to evaluate whether the impact of EG95 vaccination against transmission of *E. granulosus* s.l. in the field trials would be effective in the Iran.

Conflict of interests

None.

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